610.5
A595
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v. 32
Suppl. no. 5

ANNALES MEDICINAE EXPERIMENTALIS ET BIOLOGIAE FENNIAE

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# A SERUM LABILITY PHENOMENON

## INVOLVING ETHANOL PRECIPITATION AND DISSOLUTION OF THE PRECIPITATE IN SULPHURIC ACID

BY

E. J. JOKINEN

VOL. 32 1954 SUPPLEMENTUM 5

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# A SERUM LABILITY PHENOMENON

## INVOLVING ETHANOL PRECIPITATION AND DISSOLUTION OF THE PRECIPITATE IN SULPHURIC ACID

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# PREFACE

I wish to extend my sincere thanks to my teacher, Professor K. O. Renkonen, M.D., head of the Department of Serology and Bacteriology, University of Helsinki, who suggested the subject of this investigation. He has always been ready to give valuable advice and has never failed to find time to encourage me at every stage of my work. In his Department I have enjoyed the most favourable working conditions and received all the technical help available.

I wish to thank Professor I. Vartiainen, M.D., head of the Second Medical Clinic, University of Helsinki, for many blood samples and for the opportunity of carrying out electrophoretic analyses, and also for his kindness in placing the case histories at my disposal.

The material of rheumatic diseases comes to a larger extent from Kivelä Hospital. It gives me pleasure to record here my indebtedness to its chief, Professor P. Soisalo, M.D.

I also wish to thank the chiefs of the various hospitals for their kindness in permitting me access to case histories, and Dr. H. Nevanlinna, M.D., head of the Blood Bank of the Finnish Red Cross, for the samples of normal material.

The whole staff of the Department of Serology and Bacteriology has always been ready to help me. I wish to mention particularly Mrs. Ruth Elo, Dispensing Chemist, who has assisted me in the technical work.

I also wish to thank Mrs. Hilkka Kontiopää, M.A., and Mr. L. A. Keyworth, M.A. (Cantab), for the translation of my paper into English.

Valuable financial aid for this work was received from Reumaliitto ry (Association for Rheumatic Diseases).

Helsinki, April 1954.

*E. J. J.*

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# INTRODUCTION

The present investigation is the result of Professor K. O. Renkonen's suggestion in 1951 that I should start experiments to evolve a method of detecting the difference between normal serum and the sera of subjects suffering from rheumatic diseases.

The starting point for the experiments was an effort to precipitate the polysaccharides of the serum with alcohol together with proteins. It was hoped that treating the precipitate with concentrated sulphuric acid would produce a correlation between the darkening of the solution and the polysaccharide content of the serum. The method did actually show a difference between rheumatic and normal sera. It was also discovered that cortisone treatment distinctly diminished this difference (12).

By the time the preliminary reports had been published, however, it seemed quite evident that it was not the difference in the polysaccharide content but the protein changes in the sera that really accounted for the phenomenon. The study and development of the technique used, the investigation of an extensive clinical material, and comparison of the results with those attained by electrophoresis (14) confirmed this assumption and showed that the phenomenon observed should be classed as a serum lability phenomenon.

# TECHNIQUE

## TECHNIQUE OF A SINGLE TEST

2.5 ml of 94 per cent ethyl alcohol was measured into a test tube, inside diameter 11—12 mm. 0.25 ml of serum was pipetted into it at room temperature, a drop at a time, the drops directed straight onto the alcohol without touching the sides of the tube. The tube was placed, without shaking, in a refrigerator at +4° C, where it was allowed to stand unsealed for 24 hours. Then it was centrifuged at 2500 r.p.m. for 5 minutes in an angle head centrifuge, the test tube at an angle of 45° to the rotation plane; the rotation radius was 10 cm. The supernatant was discarded and the tube stood upside down at room temperature for 15 min. 1 ml of 95 per cent sulphuric acid was now added to the precipitate and allowed to act for 5 min. The reading was taken.

The result was considered negative (—) when the precipitate remained completely undissolved. In such a case the sulphuric acid remained colourless and did not warm up. The more positive the result the more the precipitate dissolved, the hotter the sulphuric acid grew and the darker brown it became. The result was taken as weakly positive (±) if some of the precipitate clearly dissolved and a weak brown colour developed, and strongly positive (+) if the precipitate was completely dissolved and a strong brown colour formed.[1]

## SOURCES OF ERROR

There proved to be a number of factors capable of affecting decisively the final result. A minimum of ten sera was employed in

[1] In some cases a special symbol (—?) was used for a result that was considered negative but was actually just bordering on weakly positive (±). In such cases the dissolution of the precipitate and the development of the brown colour was minimal. This symbol was only employed in the »tentative material» because of the few readings in each case.

each case to study the various sources of error, and the »correct» technique and the one under investigation were applied parallelly.

It was found that the serum to be studied must contain no erythrocytes; the presence of erythrocytes or a previous haemolysis gave a false positive result. The most reliable method proved to be centrifuging the studied sample twice to ensure the complete removal of erythrocytes.

The blood sample must be taken from patients with an empty stomach, preferrably in the morning. A sample taken after a meal easily gave a false positive result.

It was essential to pipette the serum under investigation drop by drop straight into the alcohol. If the serum was allowed to flow down the sides of the test tube some of it travelled right down to the bottom and normal precipitation failed when the layer of precipitate on top of the serum isolated it from the alcohol. Although the contents of the tube were later mixed by shaking and complete precipitation followed it was easy to get a false positive result in such cases. If again all the serum was blown into the alcohol simultaneously divergent results were obtained at different times from one and the same sample.

The precipitate must stand in alcohol for 24 hours in order to ensure as marked a difference as possible between the positive and negative modes of reaction. Centrifugation and the addition of sulphuric acid immediately after precipitation led to the dissolution of all the precipitates in the sulphuric acid, i.e. in negative cases a false positive result was obtained. A precipitate giving a negative result, therefore, seemed gradually to become less and less soluble in the course of 24 hours than a precipitate giving a positive result. The difference, it is true, emerged after a few hours, but a standing time of 24 hours was adopted to give the difference its maximum accentuation. A period exceeding 24 hours, again, seemed to detract from the result in that a positive precipitate became somewhat less readily soluble.

With a centrifuge in which the longitudinal axis of the test tube coincided with the rotation plane the precipitate packed itself in the deepest part of the tube bottom. With the test tube at an angle of 45° to the rotation plane the precipitate accumulated at a slightly higher level. In the former case the effect of the sulphuric acid on the precipitate was weaker, and the difference between

a positive and a negative mode of reaction was somewhat less marked.

When the tube was turned upside down after centrifugation care had to be taken not to let it fall from the rack; jarring might have loosened the precipitate from the bottom of the tube. When this occurred the precipitate rose to the surface easily after the addition of sulphuric acid and its dissolution was retarded.

The moisture content of the precipitate before the addition of sulphuric acid proved to be a very important factor. The more the precipitate was allowed to dry the less readily it dissolved even in a positive case, becoming finally completely insoluble at room temperature. Thus the difference between positive and negative decreased and finally disappeared. It the tube was allowed to stand upside down longer than for 15 min., or was kept e.g. in sunlight or otherwise at too high a temperature the precipitate became too dry. If again too short a time was allowed with the tube reversed the supernatant did not have time to flow out and the balance remaining in the tube affected the sulphuric acid action adversely. For these reasons a drying time of 15 min. at room temperature seemed most suitable.

In all the other stages too temperature distinctly influenced the final result. If the temperature of the serum specimen and the alcohol on precipitation was high the resulting precipitate dissolved less readily than that obtained at a lower temperature. A positive serum, in such a case, could give a negative result. On the other hand cooling the serum and the alcohol to a temperature below room temperature did not seem to contribute towards improving the results sufficiently to warrant complicating the method.

As pointed out above, no difference in solubility existed between the positive and the negative precipitate immediately on precipitation; the difference developed gradually while the precipitates were kept in alcohol. The higher the temperature at which they were kept the more rapidly the difference developed but the smaller it remained finally. The best result was attained by keeping the precipitates in a refrigerator although this required a wait of 24 hours to achieve the maximal difference.

The temperature after the addition of sulphuric acid seemed to be another factor of importance. At room temperature the negative precipitate remained undissolved and only the positive dissolved.

The higher the temperature was raised the more rapidly the positive precipitate dissolved and the stronger was the brown colour formation; but at the same time the negative precipitate also began to dissolve. The difference between them was no longer absolute but only relative. Lowering the temperature, again, resulted in an increasingly poor dissolution of the positive precipitate and hence a reduction in the said difference. If the heat that developed in the dissolving process was prevented from escaping, e.g. by lining the tube with some insulating substance, the temperature of the sulphuric acid attained higher values than otherwise. In a positive case this resulted in a more rapid dissolution of the precipitate and the development of a stronger brown colour, but it also led to a dissolution of the precipitate in a negative case. This showed that heat evidently developed even when the result was negative, but so little that it had time to escape from an uninsulated tube kept in the rack at room temperature.

The mode of reaction of a positive serum was found to undergo a change towards the negative if the serum was kept unfrozen. At room temperature this change was observable, in a weak form, as soon as after some 48 hours; but if stored in a refrigerator at +4° C there was no change until after approximately a week. If stored in a deepfreeze at —20° C the mode of reaction of the serum was found to keep well for 2—5 months.

## MODIFICATIONS OF THE TECHNIQUE

Several different modifications were tested in the various phases in an effort to improve the technique. A number of them have been reported on above in the discussion of sources of error. Of the others mention may be made of the following.

Apart from 94 per cent alcohol, the following substances were employed for precipitation: (1) other alcohols, (2) acetone, (3) trichloracetic acid and (4) copper sulphate solution.

As regards the alcohols, the higher the molecular weight the weaker was their capacity to precipitate. With methyl alcohol the difference between the positive and the negative mode of reaction emerged practically as distinctly as with ethyl alcohol. With acetone too the difference was very distinct; but acetone was poorly suited

to the purpose as, with the reaction temperature rising, it alone too readily gave the sulphuric acid a brown colour.

When trichloracetic acid or copper sulphate solutions of various strengths were used for precipitation no differences were observed between the different sera.

The heat developing from the action of precipitate dissolving in sulphuric acid grows as the positivity of the result increases. An attempt was made, therefore, to obtain a more accurate reading by measuring the temperature of the sulphuric acid than had been possible by mere ocular evaluation of the dissolution or by colour assessment. Firstly, the meter employed must be sensitive enough to indicate the rapid temperature changes of the sulphuric acid. Secondly, it must have the smallest possible thermal capacity; and thirdly, it must be proof against the corrosive effect of sulphuric acid. A sensitive galvanometer was employed for the purpose, the ends of the leads to be placed in the sulphuric acid having been glass coated. The temperature was found to vary considerably in the different levels of the sulphuric acid; on the surface of the precipitate it was generally some 10—15° C higher than elsewhere. The temperatures corresponding to the various ocular readings are given in Table 1 (the highest temperature after sulphuric acid addition, measured on the precipitate surface).

TABLE 1

THE HIGHEST TEMPERATURE OF SULPHURIC ACID CORRESPONDING TO VARIOUS OCULAR READINGS

| Reading | Temperature ° C |
|---|---|
| — | —61 |
| —? | 62—69 |
| ± | 70—78 |
| + | 79— |

As a rule it was possible, in any two cases where the difference in temperature was a couple of degrees only, to observe ocularly even a difference in the dissolution of the precipitates and in the intensity of the brown colour that developed. As reported elsewhere, the unintentional variations in the technique involved such great potential errors that it seemed unnecessary to take the more

accurate temperature reading of the result, particularly as this entailed considerable difficulties when several samples were handled at a time.

Colorimetric determination of the intensity of the brown colour also seemed too meticulous a method considering the said sources of error. In so far as these sources of error could be eliminated by further developing the technique both of the above methods might prove useful.

## RESULTS ON REPEATING THE TEST

A number of the sources of error involved in the technique employed have been discussed above. In addition to these known sources of error there are apparently others, unknown so far. Hence it can be assumed that readings obtained from one and the same serum at different times will vary somewhat. An attempt was made to study the extent of this variation as follows.

*Material.* — The sera of 200 hospital patients were investigated. The diagnosis of most of them was rheumatoid arthritis or rheumatic fever.

*Technique.* — Efforts were made to follow the technique described above as closely as possible. The investigation was carried out on a minimum of two consecutive days, twice each day, i.e. each sample was tested a minimum of four times. The sera were kept in a refrigerator at + 4° C.

*Results.* — In the attached Table 2 the cases are classified as follows:

Group A: all four readings identical.

Group B: one of the readings deviates from the others but by no more than one degree.

Group C: two identical readings deviate from the others but by no more than one degree.

Group D: others.

TABLE 2

EVALUATION OF THE FINAL RESULT ON THE BASIS OF READINGS

| Group | Readings | Final Evaluated Result | | Number | % |
|---|---|---|---|---|---|
| A | + + + + | + | | 15 | 7.5 |
| | ± ± ± ± | ± | | 17 | 8.5 |
| | — — — — | — | | 64 | 32.0 |
| | | | Total | 96 | 48.0 |
| B | + + + + | + | | 7 | 3.5 |
| | + ± ± ± | ± | | 14 | 7.0 |
| | ± ± ± — | ± | | 8 | 4.0 |
| | ± — — — | — | | 24 | 12.0 |
| | | | Total | 53 | 26.5 |
| C | + + ± ± | + | | 12 | 6.0 |
| | ± ± — — | ± | | 31 | 15.5 |
| | | | Total | 43 | 21.5 |
| D | + + — — | | | 1 | 0.5 |
| | + ± ± — | | | 2 | 1.0 |
| | + ± — — | | | 1 | 0.5 |
| | + + ± — | | | 4 | 2.0 |
| | | | Total | 8 | 4.0 |

Table 3 compares the results of the different days in Group C. The readings of one and the same day tend to show a deviation in the same direction. This type accounts for some two-thirds of all the readings.

TABLE 3

DEVIATION OF THE READINGS ON DIFFERENT DAYS IN GROUP C OF TABLE 2

| Readings | | Number |
|---|---|---|
| 1st Day | 2nd Day | |
| + + | ± ± | 4 |
| ± ± | + + | 4 |
| ± ± | — — | 14 |
| — — | ± ± | 8 |
| + ± | + ± | 4 |
| ± — | ± — | 9 |

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Another two tests were made in 13 cases where four negative readings had been obtained. All the readings persisted negative.

Of the cases in Group D, 6 samples sufficed for more than four tests. The readings obtained are recorded in Table 4.

TABLE 4

EVALUATION OF THE FINAL RESULT ON THE BASIS OF ADDITIONAL TESTS

| Case | Readings | | Final Evaluation of the Result |
|---|---|---|---|
| | Original Tests | Additional Tests | |
| 1 | + + ± — | + + | + |
| 2 | + + ± — | ± ± | ± |
| 3 | + + ± — | — — — — | — |
| 4 | + ± ± — | — — | — |
| 5 | + ± — — | ± — — | — |
| 6 | + ± — — | — — — | — |

*Discussion.* — Assessment of the final result on the basis of individual readings obtained by repeating the test can be seen from Table 2.

On repeating the test on a given serum the readings reveal so many minor variations that a certain potential error must be considered inherent in a single reading. The same applies to two readings, particularly if the tests are carried out at one time because the readings then tend to deviate in the same direction.

It is evident that if the mode of reaction of a serum lies at the margin of two readings even a small deviation in technique or evaluation of the reaction will suffice to shift the case to one side or the other. The influence of technical errors can be reduced by repeating the test.

A total of four readings spread over two consecutive days gives a relatively reliable result in approx. 96 per cent of the cases (Groups A, B and C).

Clearly the Group D readings are unreliable. Provided the necessary serum is available additional investigations should be made to find the correct result (Table 4). However, it must be borne in mind that a positive reaction may be weakened if serum is kept too long at room temperature, as was pointed out in discussing the sources of error. The most reliable procedure in the cases of this group is to obtain a new sample.

*Summary.* — When the test was carried out on 200 serum samples twice on two consecutive days, i.e. a total of four times, using the technique described in the chapter headed »Technique of a single test», the reading obtained was the same every time in 48 per cent of the cases. In another 48 per cent the readings deviated from one another by one degree. In the balance of 4 per cent the deviations were greater.

*In investigating the phenomenon under review the test in each case, unless otherwise mentioned, has been carried out twice on each of two consecutive days, i.e. a total of four times, using the technique described under the heading »Technique of a single test». In so far as the readings have been mutually identical or deviated by one degree only the result has been evaluated on the basis of Table 2. In the other cases the tests have been repeated.*

# CLINICAL MATERIALS

## COLLECTION PLAN

The following considerations governed the collection of the serum samples:

(A) The reaction with normal material.

(B) Discovery of the diseases and conditions in which reactions differing from the normal occur.

(C) A more detailed study of the behaviour of the phenomenon under investigation in some such disease or group of diseases.

For (B) a so-called tentative material was collected and for (C) the material of rheumatic diseases.

## NORMAL MATERIAL

*Material.* — The bulk of the normal material consisted of the sera of 129 blood donors. They were not selected in any way. This material is the one reported on by the writer in his previous investigation into rheumatic diseases (13). The ages of the blood donors varied from 18 to 65 years. In addition the sera of 15 persons on the laboratory staff who had reported themselves to be completely healthy, and the sera of 20 children $^1/_2$—12 years old were examined.

*Technique.* — The technique by which each individual test was carried out is described above. Each sample was examined at least twice on two consecutive days, i.e. a total of not less than four times.

*Results.* — A completely negative result was obtained each time with each serum examined, i.e. there was not a single even weakly positive reading in the total material.

*Discussion.* — Considering the size of this material and the fact that no positive reading was obtained it can probably be taken as fairly definite that a healthy subject generally reacts negatively.

A positive result, therefore, indicates that the serum concerned is pathological, provided errors in technique can be excluded.

*Summary.* — The sera of 164 healthy subjects gave a negative reading every time. Each of them was tested a minimum of four times.

## TENTATIVE MATERIAL

*Collection of Sera.* — The vast majority of this material consisted of samples submitted to the University Department of Serology and Bacteriology, Helsinki for syphilis tests. A few dozen samples submitted for other serological reactions were also studied. The total number of samples was 1307. The preliminary results with 1263 of these cases were published in the earlier investigation into rheumatic diseases by the author (13). The material covered all University of Helsinki clinics and the municipal surgical and medical department of Maria Hospital. The section dealing with skin diseases, however, is published separately (23). All these hospitals submit a blood sample of every patient for syphilis reactions. The samples were not selected in any way, and all samples not exhausted by the serological routine tests were studied daily. However, the number of samples of eye and ear, nose and throat diseases was cut down to a minimum as the possibility of serum changes in such illnesses was considered fairly small. Similarly, the rheumatic cases were excluded as a more comprehensive rheumatic material was collected serparately.

*Technique.* — The technique for the individual test was described above.

Often the syphilis and other routine tests left but a rather small amount of the sera. For this reason a single test was considered enough in cases that gave a negative result. If a positive result was arrived at the test was repeated on the following day provided there was sufficient serum. It was seldom possible to make more than two tests. Because of the few readings efforts were made to assess the mode of reaction with the greatest possible accuracy. The —? readings were also noted separately for this reason.

*Sources of Error.* — In the early stages of collecting this material the sources of error were not quite as well known as has been reported in the section dealing with them. The results of that

period are therefore not as dependable as later on. However, the difference is so small that all the cases have been treated together in the following. This procedure was adopted because the object with this material was only to study the type of cases in which positive readings occur, and hence efforts were made to make the material as comprehensive as possible.

A source of error which probably occurred in individual cases throughout was haemolysis or the occurrence of red cells in the serum. Although an endeavour was made to pick out such samples sufficiently weak haemolysis and a small amount of red cells may have passed unnoticed. Another source of error persisting throughout the material derived from the fact that due to the routine tests the sera were over 24 hours old before they were investigated, and for a considerable part of this time they had been kept at room temperature. Although subsequently they were kept in a refrigerator there was probably a slight weakening in the positivity in the renewed tests. A possible third source of error lies in the time of sampling. Some samples may have been taken after the patient in question had had a meal although the hospital routine is to take blood samples from fasting patients.

Hence the tentative material had several inherent weaknesses. Obviating every source of error, however, would have necessitated some other method of collection, and it would have been exceedingly difficult to make the material equally comprehensive and clinically many-sided.

*Results.* — 90 out of the 1307 sera studied, i.e. 6.9 per cent, gave positive readings. Their breakdown by the medical specialties is recorded in Table 5.

The 90 cases giving positive readings included 43 with only one diagnosis. Others may have had quite a number. Not all the diagnoses, however, were considered in compiling the list (Table 6) of the diseases or conditions in which positive readings may occur; efforts have been made to note each case under a single principal diagnosis. This procedure was adopted because the other diagnoses of a considerable number of patients could not be considered with any probability to be related to the phenomenon under review. On listing the cases of this type and those with a single diagnosis it was found that the majority fell into definite disease groups of a certain type. For this reason an attempt was made, in the

TABLE 5

THE BREAKDOWN OF CASES IN THE TENTATIVE MATERIAL GIVING POSITIVE READINGS IN DIFFERENT BRANCHES OF MEDICINE

| | Number of Cases | | % |
|---|---|---|---|
| | Total | Positive | |
| Internal medicine ..... | 609 | 62 | 10.2 |
| Surgery ..... | 293 | 14 | 4.7 |
| Gynaecology and obstetrics .... | 206 | 6 | 2.9 |
| Paediatrics ..... | 84 | 2 | 2.4 |
| Psychiatry and neurology .... | 55 | 3 | 5.5 |
| Radiotherapy..... | 41 | 3 | 7.5 |
| Ophthalmology ..... | 13 | — | — |
| Oto-rhino-laryngology ..... | 6 | — | — |
| Total | 1307 | 90 | 6.9 |

remaining minority also, to decide on the principal diagnosis and to group the cases accordingly. In the review of the results these cases have in each instance been reported on in detail where necessary.

The corresponding negative diagnoses also have been entered, each under a single diagnosis, following the procedure described above.

In Table 6 the headings of the columns have not been repeated after each new diagnosis.

In the column »Subsidiary diagnoses» those present somewhere else in the list as principal diagnoses giving positive results but not considered as such in these particular cases are marked • .

In Table 6 are entered the results of the erythrocyte sedimentation rate (Westergren's method), examinations of the serum proteins and some serum lability reactions widely used, provided a sample had been taken for these purposes within three days of taking the sample submitted to the University of Helsinki, Department of Serology and Bacteriology. Furthermore, the »Additional data» column contains data, picked out from hospital journals, that may possibly account for the result of the test in the case concerned.

The fraction entered after each diagnosis indicates the positive cases in relation to the total number of cases.

In 20 of the cases giving positive readings listed in Table 6 a serum protein analysis or one or several serum lability reactions

TABLE 6

CASES GIVING POSITIVE READINGS IN TENTATIVE MATERIAL

| Case | Readings 1st Day | Readings 2nd Day | Readings 3rd Day | Subsidiary Diagnoses | SR | Additional data |
|---|---|---|---|---|---|---|
| | | | | *Diseases of the liver and gallbladder* | | |
| **Cirrhosis hepatis 5/6** | | | | | | |
| 1 | + | + | | Arteriosclerosis<br>Hypothyreosis<br>Diverticula duodeni<br>Colitis • | 23 | Total protein 7.0, alb 2.5, glob 4.5 |
| 2 | + | + | | Anaemia sec.<br>Hypertensio arterialis<br>Insufficientia cordis | 13 | Total protein 5.5<br>Stolte+ Thymol+ |
| 3 | + | + | | Status p. cholecystect. | 97 | |
| 4 | ± | ± | | Haemorrhagia ex ani<br>Anaemia posthaemorrh.<br>Hypertensio arterialis<br>Cardiosclerosis<br>Achylia gastrica | 17 | Stolte— Thymol+ |
| 5 | ± | ± | | Abusus etanoli •<br>Constitutio psychopathica | 89 | Takata+ Stolte—<br>Thymol+ |
| **Hepatitis acuta 5/7** | | | | | | |
| 1 | ± | —? | | — | 3 | Stolte— Thymol+ |
| 2 | ± | —? | | — | 17 | Stolte+ Thymol+ |
| 3 | ± | — | | — | 37 | Stolte— Thymol— |
| 4 | ± | | | — | 39 | Takata+ Stolte+<br>Thymol+ |
| 5 | ± | | | — | — | |
| **Cholecystitis (or cholecystopathia) 2/20** | | | | | | |
| 1 | + | —? | | Myodegeneratio cordis | 51 | |
| 2 | ± | + | | — | 98 | |
| **Cholelithiasis 2/20** | | | | | | |
| 1 | + | ± | | — | 26 | Stolte— |
| 2 | + | —? | | Susp. tumoris (benign.) ventriculi | 11 | Takata— Stolte ±<br>Thymol— |
| | | | | *Diseases of collagen* | | |
| **Erythema nodosum 2/2** | | | | | | |
| 1 | ± ± ± | + ± | + + | — | 100 | |
| 2 | ± —? | —?—? | | Febris rheumatica? • | 62 | |
| **Lupus erythematosus disseminatus 2/2** | | | | | | |
| 1 | + | —? | | — | 90 | |
| 2 | ± | —? | | Anaemia hypochromica sec.<br>Hepatomegalia<br>Ascites | 156 | |

| Case | Readings | | | Subsidiary Diagnoses | SR | Additional data |
|---|---|---|---|---|---|---|
| | 1st Day | 2nd Day | 3rd Day | | | |
| | | | | *Diseases of the blood* | | |
| **Lymphogranulomatosis maligna 4/6** | | | | | | |
| 1 | + | | | — | 103 | |
| 2 | ± | ± | | — | 60 | |
| 3 | + —? —? | ± —? | | — | 29 | |
| 4 | + | — | | — | 77 | |
| **Leucaemia monocytica 1/2** | | | | | | |
| 1 | + + | — — | — — | Thromboangiitis obliterans • | 111 | |
| **Leucaemia myeloides 1/2** | | | | | | |
| 1 | ± | — | — | — | 70 | |
| **Myeloma 2/2** | | | | | | |
| 1 | + | + | | Status p. fractur. colli fem.<br>Venectasiæ haemorrhoidales | 143 | Total protein 15.7 |
| 2 | —? —? | ± —? | | — | 166 | Takata+ Stolte+ Thymol+ |
| **Panmyelophthisis 1/2** | | | | | | |
| 1 | ± | —? | | Pneumonia hypostatica • | 43 | Stolte+ Thymol— |
| | | | | *Diseases of the kidney* | | |
| **Nephritis acuta 2/6** | | | | | | |
| 1 | ± | + | | — | 67 | Total protein 3.9 |
| 2 | ± | ± | | — | 26 | |
| **Nephritis chronica 3/8** | | | | | | |
| 1 | + | + | | Myodegeneratio, dilatatio et insufficientia cordis<br>Hypertrophia prostatae | 71 | |
| 2 | + | + | | Anaemia | — | |
| 3 | + | —? | | Gastritis anacida<br>Varices extremit. inf. | 16 | |
| **Degeneratio polycystica renuum 1/2** | | | | | | |
| 1 | + | ± | | — | 64 | |
| **Tuberculosis renis 1/1** | | | | | | |
| 1 | + | + | | — | 104 | Electrophoresis: alb 3.49, $\alpha_1$ 0.62, $\alpha_2$ 1.43, $\beta$ 1.69, $\gamma$ 1.18 |
| **Tumor renis 2/4** | | | | | | |
| 1 | + | + | | — | 124 | |
| 2 | ± | — | | Hypertensio arterialis<br>Myodegeneratio et dilatatio cordis | 75 | |

| Case | Readings | | | Subsidiary Diagnoses | SR | Additional data |
|---|---|---|---|---|---|---|
| | 1st Day | 2nd Day | 3rd Day | | | |
| *Infections (other than those mentioend above)* | | | | | | |
| **Abscessus pulmonis 1/1** | | | | | | |
| 1 | + | | | Anaemia secundaria | 60 | Total protein 6.0, alb 3.2, glob 2.8 |
| **Appendicitis acuta 1/33** | | | | | | |
| 1 | ± | | | — | — | |
| **Colitis 2/14** | | | | | | |
| 1 | + | — | | Febris e causa ignota | 120 | Total protein 9.6. Takata— Stolte— |
| 2 | —? | ± | | Achlorhydria gastrica<br>Hypertensio arterialis | 22 | |
| **Endocarditis lenta 1/3** | | | | | | |
| 1 | ± | | | Stenosis ostii aortae | 33 | |
| **Mastitis purulenta 1/2** | | | | | | |
| 1 | ± | | | — | 89 | |
| **Parametritis acuta 1/2** | | | | | | |
| 1 | + | + | | Cysta glandulae Bartholini | 148 | |
| **Pneumonia 2/30** | | | | | | |
| 1 | + | + | | Syphilis non medicata | 104 | |
| 2 | ± | | | Tuberculosis pulm?<br>Bronchiectasiae? | 111 | |
| **Spondylitis tuberculosa 1/1** | | | | | | |
| 1 | ± | ± | ± | Abscessus congestionis retroperitonealis<br>Necrosis (compressiva?) renis<br>Anomaliae congen. extrem. inf. l.a. | 70 | |
| **Tuberculosis pulmonis 2/22** | | | | | | |
| 1 | + | | | — | 63 | |
| 2 | ± | | | Arteriosclerosis<br>Bronchitis chronica<br>Anaemia hyperchromica | 67 | Total protein 6.3 |
| **Thrombophlebitis 1/7** | | | | | | |
| 1 | ± | | | Res. p. embol. cerebri?<br>Cholecystopathia?<br>Anaemia normochromica levis | 53 | |
| **Tonsillitis acuta 1/7** | | | | | | |
| 1 | ± | | | Anaemia secundaria | 23 | |

| Case | Readings | | | Subsidiary Diagnoses | SR | Additional data |
|---|---|---|---|---|---|---|
| | 1st Day | 2nd Day | 3rd Day | | | |
| | | | | *Pregnancy and its complications* | | |
| Graviditas m. X 1/8 | | | | | | |
| 1 | ± | —? | | — | — | |
| Abortus 1/32 | | | | | | |
| 1 | + | | | — | 58 | |
| Graviditas extrauterina 1/7 | | | | | | |
| 1 | + | ± | | — | 50 | The sample was taken 3 days after the operation |
| Nephrogestosis 1/6 | | | | | | |
| 1 | + | —? | | — | — | |
| | | | | *Diseases of the thyroid gland* | | |
| Struma nodosa 2/20 | | | | | | |
| 1 | + | — | | — | 9 | |
| 2 | ± | | | Graviditas m. III<br>Hypertrophia cordis<br>Varices cruris | 17 | |
| Thyreotoxicosis 3/26 | | | | | | |
| 1 | + | ± | | Struma nodosa •<br>Bronchitis asthmatica<br>Neurosis | 32 | |
| 2 | + | + | | — | 23 | |
| 3 | + | + | + | Struma nodosa •<br>Cholecystopathia • | 15 | Stolte— Thymol+ |
| | | | | *Cardiovascular diseases (other than those mentioned above)* | | |
| Angina pectoris 1/16 | | | | | | |
| 1 | ± | —? | | Emphysema pulmonum<br>Cor pulmonale incipiens | 3 | |
| Calcificatio pericardii 1/1 | | | | | | |
| 1 | ± | ± | | Ulcus ventriculi • | 92 | Total protein 6.7, alb 3.7, glob 3.0<br>Stolte— Thymol— |
| Dystonia neurocirculatoria 1/25 | | | | | | |
| 1 | ± | | | — | 7 | |
| Infarctus cordis 1/14 | | | | | | |
| 1 | + | + | | Pyelonephritis | 48 | The knee had been swollen for four weeks |
| Thromboangiitis obliterans 1/7 | | | | | | |
| 1 | ± | ± | | Status p. sympathectomiam<br>Hypertensio arterialis | 10 | |

| Case | Readings | | | Subsidiary Diagnoses | SR | Additional data |
|---|---|---|---|---|---|---|
| | 1st Day | 2nd Day | 3rd Day | | | |
| | | | | *Carcinoma (in various organs) 10/62* | | |
| 1 | + | + | + | Carcinoma solidum hepatis et vertebrae (metastases) | 97 | Total protein 7.1 |
| 2 | + | + | | Metastases oss. ilei l.a.<br>Residua p. operat. radical. mammae propter ca<br>Coliuria | 100 | |
| 3 | + | ± | | Metastases columnae vertebr. et coxae<br>Paraplegia<br>Anaemia secundaria | 81 | |
| 4 | + | ± | | Metastases hepatis<br>Anaemia posthaemorrhagica<br>Cardiosclerosis<br>Hypertrophia prostatae | 28 | Stolte+ Thymol+ |
| 5 | ± | + | | Anaemia posthaemorrhagica<br>Hypoproteinaemia<br>Cardiosclerosis<br>Insufficientia cordis | 106 | Total protein 5.3, alb 1.9, glob 3.4<br>Stolte+ Thymol— |
| 6 | + | ± | | — | 21 | Total protein 5.8 |
| 7 | —? | + | | Tumor vesicae urinariae<br>Cystitis chronica | 111 | |
| 8 | ± | —? | | — | 51 | Total protein 7.1 |
| 9 | ± | | | — | 113 | |
| 10 | ± | — | | Metastases organorum variorum corporis | 86 | |
| | In addition to the groups mentioned the following diagnoses occurred | | | | | |
| Alcoholismus chronicus 1/3 | | | | | | |
| 1 | ± | | | Psychopathia | 29 | |
| Arthrosis deformans 1/3 | | | | | | |
| 1 | ± | — | | — | — | |
| Diabetes mellitus 4/27 | | | | | | |
| 1 | + | —? | | Myodegeneratio et insufficientia cordis<br>Nephrosclerosis<br>Tumor pulmonis | 5 | |
| 2 | —? | ± | | — | 18 | |
| 3 | ± | | | — | 15 | |
| 4 | ± | —? | —? | Arteriosclerosis<br>Hypertrophia prostatae<br>Syphilis medicata | 16 | |
| Epilepsia 1/10 | | | | | | |
| 1 | ± | | | Dementia et psychosis epileptica | 35 | |
| Gangraena pedis 1/1 | | | | | | |
| 1 | + | —? | | — | 60 | Strong dehydration |

| Case | Readings | | | Subsidiary Diagnoses | SR | Additional data |
|---|---|---|---|---|---|---|
| | 1st Day | 2nd Day | 3rd Day | | | |
| **Hyperglobulinaemia 1/1** | | | | | | |
| 1 | ± | + | | Cardiosclerosis<br>Angina pectoris • | 67 | Total protein 7.0, alb 3.5, glob 3.5 Stolte— |
| **Imbecillitas 1/2** | | | | | | |
| 1 | + | | | — | 15 | Total protein 7.6 |
| **Melaena 1/3** | | | | | | |
| 1 | ± | — | | Ulcus ventriculi? •<br>Arteriosclerosis<br>Anaemia posthaemorrhagica | 18 | |
| **Myalgia 1/1** | | | | | | |
| 1 | + | | | Cephalalgia extracranialis<br>Atrophia cerebri | 85 | Peritonsillar abscess was opened one week earlier |
| **Ulcus cruris 3/6** | | | | | | |
| 1 | + | + | | Eczema varicosum cruris | 10 | |
| 2 | + | + | | — | 55 | |
| 3 | ± | —? | | Diabetes mellitus • | 60 | |
| **Ulcus ventriculi (duodeni) 1/64** | | | | | | |
| 1 | ± | | | — | 28 | Strong dehydration |

had been made. Results differing from the normal had been obtained in all but 3 cases. On the other hand, in the cases giving negative readings in Table 6, 22 had been examined by the said methods and results differing from the normal had been obtained in 10 cases.

TABLE 7

COMPARISON OF SR IN THE POSITIVE AND NEGATIVE CASES IN CONDITIONS GIVING POSITIVE READINGS

| Positive Cases | | Negative Cases | |
|---|---|---|---|
| SR | Number of Cases | SR | Number of Cases |
| 0— 10 | 7 | 0— 10 | 125 |
| 11— 20 | 12 | 11— 20 | 80 |
| 21— 30 | 11 | 21— 30 | 51 |
| 31— 50 | 13 | 31— 50 | 45 |
| 51— 70 | 17 | 51— 70 | 33 |
| 71—100 | 15 | 71—100 | 27 |
| 101— | 14 | 101— | 7 |

## TABLE 8

CONDITIONS WITH ONLY A NEGATIVE REACTION, CLOSELY ATTACHED TO THOSE WITH POSITIVE READINGS

| Diagnosis | Number of Cases |
|---|---|
| **Diseases of the liver and gallbladder** | |
| Hepatomegalia | 1 |
| **Diseases of the blood** | |
| Anaemia hypochromica essentialis | 3 |
| » normochromica (symptomatica) | 20 |
| » posthaemorrhagica | 17 |
| » hyperchromica | 1 |
| » perniciosa | 2 |
| **Diseases of the kidney (and urinary passages)** | |
| Hydronephrosis | 1 |
| Nephrosclerosis | 5 |
| Nephrolithiasis | 3 |
| Ureterolithiasis | 2 |
| Pyelonephritis acuta | 3 |
| Pyelitis chronica | 8 |
| Pyelocystitis | 4 |
| Haematuria | 1 |
| **Infections (other than those mentioned above)** | |
| Laryngotracheitis acuta | 2 |
| Pharyngitis | 1 |
| Bronchitis acuta | 6 |
| Bronchitis chronica | 3 |
| Otitis media suppurativa | 4 |
| Infectio acuta | 9 |
| Influenza | 1 |
| Pertussis | 1 |
| Morbilli | 1 |
| Scarlatina | 2 |
| Mononucleosis infectiosa | 1 |
| Gastroenteritis acuta | 7 |
| Encephalitis | 3 |
| Salpingo-ophoritis acuta | 5 |
| Lymphadenitis colli | 1 |
| Tuberculosis lymphoglandularum | 1 |
| Tuberculosis sterni | 1 |
| Endometritis tuberculosa | 1 |
| Pleuritis exsudativa | 6 |
| Gonitis seropurulenta | 1 |
| Gonitis acuta | 1 |
| Morbus Reiteri | 1 |
| Febris e causa ignota | 3 |
| **Pregnancy and its complications** | |
| Graviditas m II | 4 |
| » III | 11 |
| » IV | 4 |
| » V | 3 |
| » VI | 3 |
| » VII | 1 |
| » VIII | 2 |
| » IX | 1 |
| Hyperemesis gravidarum | 4 |
| **Diseases of the thyroid gland** | |
| Struma diffusa | 2 |
| Myxoedema | 3 |
| Hypothyreosis | 1 |
| **Unclassified diagnoses** | |
| Dystrophia musculorum progressiva | 1 |
| Haematemesis | 3 |
| Hydrops genu | 1 |
| Osteoporosis ossium cranii | 1 |
| Sarcoma | 5 |
| Varices cruris | 3 |

As to the sedimentation rate, it was found to be $\leqq$ 10 in only 7 out of all the cases giving positive readings, and higher in all the others. Among the negative, on the other hand, both low and high values were frequent, as can be seen from Table 7.

The diagnoses belonging to the disease groups or closely attached to the individual principal diagnoses in Table 6 for which only negative readings occurred are listed in Table 8. All the diagnoses for each patient are taken into consideration.

In the cases recorded in Table 8 and in the balance of the tentative material that gave negative readings, there were numerous instances of different SR values ranging from 0 to over 100.

*Discussion.* — In assessing the positivity of the results it may be considered as evident that the most unreliable of the individual cases are those in which the result was $\pm$ or $\pm$, —, and that the reliability increases with the strength and number of the positive readings obtained.

Where a positive case, unreliable in its reaction as an individual case, occurs as one of many other positive cases under a diagnosis, the positivity should probably be regarded as relatively reliable. By way of example may be mentioned the hepatitis acuta cases, Nos. 3, 4 and 5. If again the case is the only positive one among several negative cases, the possibility that a false positive reading may be involved is greater the more numerous the negative cases and the weaker the positive reading. In such circumstances classifying the result as positive is uncertain. Especially clear examples of this in the material above are: appendicitis acuta, thrombophlebitis, tonsillitis acuta, angina pectoris, dystonia neurocirculatoria, arthrosis deformans, epilepsia and ulcus ventriculi (duodeni).

Another point worth noting which may lead to the incorrect entry of the diagnosis in Table 6 is that the disease or condition diagnosed is not the cause of the positive mode of reaction (the cause lies in some other undiagnosed disease or condition) and that the case is the only one listed under the diagnosis in question. Such cases may include: the myalgia case, in which the medical history and the high SR indicate an infection and perhaps a rheumatic disease; and the case of infarctus cordis, in which the medical history suggests a possible rheumatic disease.

A third possibility of this kind is that the selection of the principal diagnosis may be incorrect. This is most likely in the cases for

which a subsidiary diagnosis is given which appears in the list as one that has given positive results (indicated with •). If the case is the only one the inclusion of the diagnosis as a principal diagnosis in Table 6 may of course be disputable. Naturally it is possible that a disease or condition indicated by a subsidiary diagnosis may be the true cause of the positive reaction.

Regarding the negative cases, it must perhaps be considered probable that had several tests been made positive readings might have been obtained in some instances.

In only 3 of the 20 cases in which protein analysis and lability reactions were employed was no pathological result obtained. In one of these three even (colitis, Case 1) the increased amount of total protein observed is pathological, and in another (cholelithiasis, Case 1) one lability reaction only was employed. But this can in no way be taken to mean that a negative result indicates that the serum involved in each case is always normal with regard to proteins.

The diseases and conditions producing positive readings are generally of a kind in which serum protein changes are known to occur, expressly in the form of an increase of globulins compared with albumin, and lability reactions are known to give results differing from the normal (1, 2, 8, 16, 30). Excluding the diagnoses whose incorporation as principal diagnoses in Table 6 is a matter of dispute due to the possible unreliability of the readings, the following six only constitute an exception: graviditas extrauterina, struma nodosa, alcoholismus chronicus, imbecillitas, melaena and thrombo-angiitis obliterans. In the graviditas extrauterina case the blood sample was taken 3 days after the operation and this may account for the protein changes present in the serum. In the struma nodosa cases undiagnosed thyreotoxicosis may possibly account for the positive readings and similarly in the case of chronic alcoholism destruction of the parenchyma of the liver, in the melaena case the strong dehydration of the patient.

On the other hand, the diseases whose incorporation among those giving positive readings has been considered disputable because of the possible unreliability of the readings, also include kinds in which serum protein changes are known to occur, like thrombophlebitis, infarctus cordis and ulcus ventriculi (2, 30). In the last-mentioned case the strong dehydration may constitute and additional, partial cause of the positive mode of reaction.

It is in disease groups in which great serum protein changes and, above all, a strong relative increase in globulins are almost a rule that the strongest positive mode of reaction has generally been obtained and in which the number of positive readings is highest proportionately. Such are the diseases of the parenchyma of the liver (10/13ths of the total), diseases of collagen (4/4ths), malignant blood diseases (9/12ths) and the diseases of the kidney parenchyma (7/17ths). (It should be borne in mind that diseases of collagen include rheumatic diseases; the writer's series of these diseases will be reported on later in the present paper.) These disease groups may perhaps include also the majority of the carcinoma cases with positive readings (liver and bone marrow metastases) and the cases of kidney tumour. That a strong positive mode of reaction can be expected in the disease groups under review is supported, furthermore, by the fact that in the diseases listed as having produced nothing but negative readings they are represented only by hepatomegalia, haematuria, pyelonephritis, hydronephrosis and nephrosclerosis benigna, of which the first two, however, probably do not necessarily presuppose at least any considerable injuries of parenchyma.

As to the sedimentation rate, in only 7 of the cases that gave positive readings was it $\leqq 10$. In two of these even (angina pectoris and dystonia neurocirculatoria) the result of the test was uncertain. In addition, it was found that in the cases with strong positive readings the SR also as a rule was very high. On the other hand, an exceedingly high SR does not seem to presuppose a strongly positive reading nor, in general, an elevation of SR a positive mode of reaction.

A detailed analysis of the results for a certain diagnosis or their comparison with the results of a certain lability reaction is hardly possible with the present material. It would have necessitated a greater number of samples for each diagnosis and the testing of each sample a minimum of four times.

*Summary.* — Using the technique described in the chapter headed »Technique of a single test», the serum of 1307 hospital patients was studied. Each serum sample was usually tested 1—3 times if the first reading was positive, if negative, only once.

Positive readings were obtained in 90 cases. These were mainly cases of diseases or pathological conditions in which changes in

serum proteins are known to occur, expressly in the form of a globulin increase in proportion to albumins, and in which lability reactions are known to give results differing from the normal.

## MATERIAL OF RHEUMATIC DISEASES

*Material.* — The material of rheumatic diseases consisted of 77 sera. They derived from hospital patients with a diagnosis of rheumatic fever in 22 and rheumatoid arthritis in 55 cases. Their age varied between 15 and 70 years. The rheumatic disease was the sole disease the patients suffered from, or else their other diseases were such that, on the basis of the tentative material, they could be considered not to affect the mode of reaction. None had been treated with cortisone or ACTH for a minimum of 6 months before admission to hospital.

This material included the 42 cases reported on in a previous publication by the author (13).

*Technique.* — The technique by which the individual test was carried out has been reported on above. Each serum was tested at least twice on two consecutive days, i.e. a minimum of four times in all. The results take into account the final assessment only.

*Results.* — The percentages of the positive and negative results are shown in Table 9.

TABLE 9

RESULTS IN VARIOUS RHEUMATIC DISEASES

| Diagnosis | Number of Cases | Positive | | Negative | |
|---|---|---|---|---|---|
| | | Number | % | Number | % |
| Rheumatic fever .... | 22 | 16 | 73 | 6 | 27 |
| Rheumatoid arthritis | 55 | 39 | 71 | 16 | 29 |
| Total | 77 | 55 | 71 | 22 | 29 |

The relationship between the results and the erythrocyte sedimentation rate (Westergren's method) is shown in Table 10.

Table 11 shows the relationship between the mode of reaction and fever. The temperature has been taken into account for a day before and five days after the taking of the sample. Considered as

TABLE 10

EFFECT OF THE SEDIMENTATION RATE ON THE RESULTS

| SR | Rheumatic Fever | | Rheumatoid Arthritis | | Total | |
|---|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | Positive | Negative |
| — 15 | — | 2 | — | 3 | — | 5 |
| 16— 25 | — | 2 | 2 | 6 | 2 | 8 |
| 26— 40 | 2 | 2 | 8 | 4 | 10 | 6 |
| 41—100 | 6 | — | 26 | 3 | 32 | 3 |
| 101— | 8 | — | 3 | — | 11 | — |
| Total | 16 | 6 | 39 | 16 | 55 | 22 |

afebrile are the patients for whom all the readings were below +37° C. It may be mentioned that the highest recorded temperature in the material was +39.1° C.

TABLE 11

EFFECT OF FEVER ON THE RESULTS

| | Rheumatic Fever | | Rheumatoid Arthritis | | Total | |
|---|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | Positive | Negative |
| Afebrile .. | 5 | 2 | 16 | 11 | 20 | 13 |
| Febrile .... | 11 | 4 | 23 | 5 | 35 | 9 |
| Total | 16 | 6 | 39 | 16 | 55 | 22 |

The relationship between the strength of the positive results, the erythrocyte sedimentation rate and fever is shown in Table 12.

TABLE 12

DEPENDENCE OF THE STRENGTH OF THE POSITIVE REACTION ON THE SEDIMENTATION RATE AND FEVER

| Final Result | Rheumatic Fever | | | | | | | Rheumatoid Arthritis | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of Cases | SR | | | | Afebrile | Febrile | No. of Cases | SR | | | | Afebrile | Febrile |
| | | —25 | 26—40 | 41—100 | 101— | | | | —25 | 26—40 | 41—100 | 101— | | |
| — | 4 | — | 1 | 2 | 1 | 2 | 2 | 8 | — | — | 8 | — | 2 | 6 |
| ± | 9 | — | 1 | 4 | 4 | 3 | 6 | 24 | 2 | 8 | 13 | 1 | 12 | 12 |
| + | 3 | — | — | — | 3 | — | 3 | 7 | — | — | 5 | 2 | 2 | 5 |

Regarding the cases of rheumatic fever it may be mentioned, moreover, that the joints had been roentgenologically examined in 4 negative and 6 positive cases. Among the former not a single case showed changes caused by arthritis but there were three such cases in the latter. Not a single negative case of rheumatic fever revealed symptoms of carditis on auscultation whereas seven positive cases did. No definite correlation with the results was observable as regards electrocardiogram changes.

It may be pointed out, further, that the results did not seem to depend in any way on the duration of the disease.

*Discussion.* — The results with this more extensive material are largely identical with those obtained by the writer from his previous investigation into rheumatic diseases based on a smaller material (13). The percentage of positive cases of the total is approximately the same, and the dependence of the results on the activity of the disease emerges clearly. In the last mentioned respect, it is true, minor differences in degree are observable. For instance, the occurrence of the positive mode of reaction specifically with patients with a high erythrocyte sedimentation rate is somewhat more definite for rheumatic fever than for rheumatoid arthritis. As regards fever the correlation is not equally marked. However, it must be borne in mind that in the present investigation the patient was considered afebrile only provided all temperature readings were under +37° C. The dependence of the results on the activity of the disease is further indicated by the observations on the roentgenological changes of the joints and on the auscultation of the heart.

The results obtained by the technique employed in the present investigation are in many respects similar to those obtained with Weltman's reaction. By neither of the methods is the positive mode of reaction by any means specific for rheumatic diseases (30). Another common feature is the dependence of the results on the activity of the disease (25). It has been found that the salicylate content of the serum does not affect the mode of reaction in Weltman's test (28). The author's earlier observations suggest that this is also true of the technique employed in the present work (13), but salicylate present in the blood does reduce the erythrocyte sedimentation rate (10).

The results given by the Waaler-Rose test in rheumatic diseases,

on the other hand, differ sharply from those obtained in the present investigation for, according to Wager (26), positive results are obtained as follows: in rheumatoid arthritis 61 per cent, rheumatic fever 3 per cent, in other diseases 2 per cent and for healthy subjects 1 per cent of the total number of cases.

In assessing the potential uses of the method described in the present work for the diagnosis of rheumatic diseases it must be pointed out that the positive reaction is far from specific for rheumatic diseases, as the tentative material shows. On the other hand again a negative result by no means excludes the possibility of rheumatism as all sufficiently slight cases and a number of other cases as well react negatively. It is possible that the method might in some individual cases prove useful in excluding osteoarthritis in the differential diagnosis.

If, on the other hand, a rheumatic disease has been diagnosed and the other diseases giving positive results can be excluded a positive reaction probably indicates that the case in question is at least above the average in severity. A negative result, again, suggests relative inactivity. The above probably holds good above all for rheumatic fever.

*Summary.* — The sera of 22 patients affected with rheumatic fever and 55 patients affected with rheumatoid arthritis were examined.

In rheumatic fever a positive result was attained in 73 per cent and in rheumatoid arthritis in 71 per cent of the total number of cases.

The result obtained in a given case was found as a rule to depend on the activity of the disease. The phenomenon was more marked in rheumatic fever than in rheumatoid arthritis.

## ON THE CHARACTER AND CAUSES OF THE PHENOMENON

Several points have emerged in the above discussion suggesting that the phenomenon under review is in its character one of the so-called serum lability phenomena.

Results differing from the normal occurred in the very diseases in which they are known to occur when serum lability reactions are used. Results differing from the normal were also obtained practically every time these reactions were made in the positive cases of the tentative material. In discussing the sources of error it was pointed out that haemolysis, the taking of the blood sample after a meal and allowing the serum to stand for some time easily give rise to false results. These factors have been found to constitute important sources of error in many lability reactions (8, 3, 6, 22).

More than ten lability reactions based on ethyl alcohol precipitation have been developed (8). The first was by Sachs and Altman in 1908 (8). Among the best known may be mentioned the reactions developed by v. Darányi (4, 5), Keller (17) and Love and Mawson (19). In addition there are numerous reactions in which ethyl alcohol is present as a component part of the precipitation reagent, e.g. those by Waldenström (27) and Sachs and Klopstock (24).

In the study of the causes of the phenomenon reported in the following efforts were made to ascertain the factor or factors in the serum which determine its mode of reaction and the cause of the differing behaviour of positive and negative precipitate in sulphuric acid.

## SERUM FACTORS AFFECTING THE MODE OF REACTION

There is ample reason to assume that the causes of the different modes of reaction are linked to the serum proteins. The results indicate that normal subjects show a negative reaction. But positive cases occurred specifically in diseases and conditions in which protein changes in the serum have been found by means of both electrophoretic analysis and precipitation (1, 2, 16, 30). This applies particularly to the changed albumin: globulin ratio. It is known that cortisone treatment has a tendency to normalize the serum proteins (11), which might account for the transformation of a positive mode of reaction into negative in the course of treatment, as has been found to occur in rheumatic diseases (12).

## COMPARISON WITH ELECTROPHORETIC PROTEIN ANALYSIS

*Material.* — This material was published previously by the writer in co-operation with Kaipainen (14). It comprised the sera of 20 hospital patients (Table 13).

TABLE 13

DISTRIBUTION OF THE CASES BY THE DIAGNOSIS MADE IN COMPARISON OF RESULTS WITH THOSE OBTAINED BY ELECTROPHORESIS

| Diagnosis | Number of Cases |
|---|---|
| Rheumatoid arthritis | 5 |
| Rheumatic fever | 7 |
| Osteo-arthritis | 4 |
| Sarcoidosis | 1 |
| Varicose veins | 1 |
| Chronic nephritis | 1 |
| Carcinoma of the lung with bone and liver metastases | 1 |
| Total | 20 |

*Technique.* — Each serum was first tested as described in the conclusion of the chapter headed »Results on repeating the test».

The electrophoretic protein analyses were then effected with Antweiler's micro-electrophoresis apparatus (2). The buffer employed was Dole's veronal, veronal-sodium buffer: ionic strength 0.12, pH 8.6. Dialyzing time was 1—2 hours. One part of the dialyzed serum was diluted with 3 parts of the buffer. In one case, with a low total protein content, the dilution 1 : 3 was used. The current was 1.8 mA, running time 18 min. Double determination was made of each serum, and the differences were always under 1.5%, the limit above which, according to Antweiler, a new analysis is required (2).

*Results.* — In Table 14 the cases are arranged in order from negative to the most strongly positive. All four readings for each case have been taken into account in determining the order.

TABLE 14

COMPARISON OF RESULTS IN 20 CASES WITH THOSE OBTAINED BY ELECTROPHORESIS

| Case | Final Result | Electrophoresis | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Total Protein | Albumin % | Globulin % | | | | Alb. : Glob. |
| | | | | $\alpha_1$ | $\alpha_2$ | $\beta$ | $\gamma$ | |
| 1 | — | 6.80 | 68.09 | 1.18 | 6.03 | 5.15 | 19.65 | 2.13 |
| 2 | — | 7.58 | 55.54 | 2.77 | 10.16 | 14.78 | 16.75 | 1.18 |
| 3 | — | 7.40 | 51.35 | 1.35 | 8.24 | 17.97 | 21.08 | 1.06 |
| 4 | ± | 6.84 | 50.58 | 4.82 | 14.04 | 10.23 | 20.32 | 1.02 |
| 5 | ± | 7.56 | 59.66 | 5.29 | 13.23 | 7.28 | 14.55 | 1.48 |
| 6 | ± | 7.82 | 53.45 | 2.94 | 7.67 | 15.86 | 20.08 | 1.15 |
| 7 | ± | 7.11 | 49.23 | 5.63 | 9.14 | 18.28 | 17.72 | 0.97 |
| 8 | ± | 8.40 | 36.19 | 6.19 | 12.86 | 15.12 | 29.64 | 0.57 |
| 9 | ± | 8.24 | 49.03 | 3.40 | 9.22 | 12.14 | 26.21 | 0.96 |
| 10 | ± | 8.07 | 43.87 | 12.02 | 22.30 | 9.54 | 12.27 | 0.78 |
| 11 | ± | 6.14 | 32.57 | 9.45 | 26.06 | 9.77 | 22.15 | 0.48 |
| 12 | ± | 8.50 | 45.65 | 4.35 | 17.88 | 11.76 | 20.35 | 0.84 |
| 13 | ± | 7.98 | 50.13 | 5.26 | 5.01 | 16.92 | 16.04 | 1.01 |
| 14 | ± | 8.38 | 50.84 | 5.73 | 8.00 | 15.87 | 19.57 | 1.03 |
| 15 | + | 7.16 | 54.05 | 6.00 | 14.66 | 8.80 | 19.23 | 1.18 |
| 16 | + | 7.14 | 39.78 | 12.04 | 14.99 | 15.69 | 17.51 | 0.66 |
| 17 | + | 7.48 | 32.62 | 6.95 | 17.65 | 5.88 | 36.10 | 0.45 |
| 18 | + | 8.40 | 29.17 | 1.07 | 7.62 | 18.10 | 44.05 | 0.41 |
| 19 | + | 5.29 | 47.64 | 7.94 | 17.01 | 11.91 | 15.50 | 0.91 |
| 20 | + | 8.45 | 45.44 | 2.37 | 16.21 | 13.96 | 22.01 | 0.83 |

| Normal Average (According to Antweiler) ..... | Alb. | $\alpha$ | $\beta$ | $\gamma$ | Alb: Glob. |
|---|---|---|---|---|---|
| | 63.20 | 9.00 | 13.70 | 14.10 | 1.71 |

*Discussion.* — The results show an abnormal alb.: glob. ratio in favour of the globulins in all the positive cases. It can even be said that the more strongly positive the reaction of the serum the lower the alb.: glob. ratio. There are exceptions, above all cases 13, 14 and 15. The result does not seem to depend, definitely at least, on any individual globulin fraction per se, though there is perhaps some correlation with $\alpha_1$ and $\gamma$ fractions.

Total serum protein in the present material, evidently has no essential effect on the result. This is generally true of lability reactions (8).

It may be considered as evident that the quantitative changes in the serum protein fractions are not in themselves enough to account for the mode of reaction but that qualitative changes are of exceedingly great importance. The observations, therefore, concur with the general findings of comparisons between the results given by lability reactions and those obtained by electrophoretic protein analysis. The effect of the quality of the proteins has emerged clearly. Some gleanings from the extensive literature on the subject are given in the following.

Wuhrmann and Leuthardt (29) found that although a positive result with Takata's reaction is only obtained provided the alb.: glob. ratio is $< 1$ the positive reaction is actually caused by the so-called Takata proteins which are pathological proteins not present in normal serum. They are contained in the electrophoretic $\gamma$ fraction and apparently also in the $\beta$ fraction. Wuhrmann and Wunderly (31) have shown that in Weltmann's reaction the $\gamma$ fraction has an effect in non-febrile diseases quite contrary to that in inflammatory processes. The significance of the quality of proteins in the cephalin-cholesterol-flocculation test is shown by the fact that while an addition of electrophoretically isolated $\gamma$ globulin to the serum investigated as a rule changes the result towards positive (15, 21) Maclagan and Bunn (20) found the effect of $\gamma$ globulin to be stronger when isolated from hepatitis serum than from normal serum. As to thymol reaction, Kunkel and Hoagland (18) have shown that a co-factor in the positive reaction is the abnormal lipoid-protein complex which in electrophoresis accompanies the $\beta$ fraction, that hepatitis $\gamma$ globulin is also a co-factor while normal $\gamma$ globulin is not, and that normal albumin inhibits a positive result but hepatitis albumin does not.

Apparently other substances in the serum than proteins also affect the mode of reaction, as several lability reactions have shown (8). This is indicated e.g. by the effect on the result of food intake and ageing of the serum, seen in studying the sources of error.

*Summary.* — The investigation covered 20 sera, of which 3 gave a negative and 17 a positive result. Electrophoretic protein analysis showed that in the positive cases globulins had increased compared with albumins and that generally this increase was more marked the more strongly positive the result. The amount of total protein did not affect the result.

## DIFFERENCES BETWEEN POSITIVE AND NEGATIVE PRECIPITATE

*Investigations.* — The investigations described in the following were effected parallelly with a minimum of 5 sera giving a positive and 5 giving a negative reaction.

Centrifuged positive precipitate was found to differ in structure trom the negative. Dark field microscopic study showed the former co have a loose appearance and the latter to be relatively more fompact. The same difference was observed on adding water to the precipitate. The positive precipitate broke up much more readily with gentle shaking than the negative. That the dissolution of a substance was not involved in the positive mode of reaction was shown by the fact that if, after the addition of water, the centrifuging was repeated and the water discarded the difference between the precipitates still emerged on the addition of sulphuric acid.

In discussing the sources of error it was pointed out that immediately on precipitation all the precipitates reacted positively and the negative precipitates became less soluble in sulphuric acid on being left to stand in a refrigerator. If the supernatant in a negative case was carefully separated immediately on precipitation no precipitation was observed in it within 24 hours. But precipitate washed with alcohol and transferred into a fresh lot of alcohol assumed a negative reaction if left to stand for 24 hours.

*Discussion.* — There are several indicators suggesting that the behaviour of the precipitate in sulphuric acid might be governed by the water and alcohol bound to the precipitate. Firstly, sulphuric

acid of high concentration has a great affinity for water and alcohol. Secondly, the study of the sources of error has revealed that when the precipitates are allowed to dry sufficiently they all become insoluble in sulphuric acid. Mixing sulphuric acid with water or alcohol generates heat. In a positive case, with the precipitate containing more water and alcohol than in a negative case, the temperature increases more, and this again results in the dissolution of the precipitate. Considerable temperature differences have been found on the surface of the precipitate in the positive and negative cases. The assumption that a positive precipitate binds more water and alcohol than a negative might be explicable from the difference, microscopically discernible, in the structure of the precipitates.

The structural difference in the protein precipitates obtained with ethyl alcohol in the various diseases has been observed e.g. by Holmgren *et al.* (9) in Gruskin's (7) »serological» test to diagnose carcinoma. They concluded that the »positive» result given in carcinoma serum by the alcohol solution of the »antigen» employed in the test, which manifested itself in the form of a precipitate coarser than that obtained in a normal serum, was due to alcohol alone. Apart from carcinoma, a precipitate coarser than the normal was obtained in many other diseases also, e.g. in rheumatism.

The original assumption, from which the development of the technique was started and according to which the factor deciding the behaviour of the precipitate in alcohol was the polysaccharides precipitating together with the proteins, evidently does not hold good.

*Summary.* — A difference in compactness is observable under the microscope between precipitates with positive and negative reactions.

There seems to be ample reason to assume that the behaviour of the precipitate in sulphuric acid is governed by the water and alcohol bound to the precipitate.

## CONCLUSIONS

A phenomenon is described which can be induced by the following technique. A certain amount of the serum to be tested is dropped at room temperature into a test tube containing 94% ethyl alcohol. The resulting precipitate is allowed to stand in alcohol at +4° C for 24 hours. After centrifuging the supernatant is discarded and the tube is left upside down for 15 min. After this sulphuric acid of high concentration is poured into the precipitate in the tube at room temperature. The reading is taken after 5 min. In a negative case the precipitate remains undissolved and the sulphuric acid colourless. In a weakly positive case the precipitate is partly dissolved and the sulphuric acid turns brown. In a strongly positive case the precipitate is completely dissolved and the sulphuric acid forms a darker brown colour.

The technique described has several sources of error and thus somewhat differing readings are obtained at different times on repeating the test with one and the same serum. When 200 sera were tested, each of them four times, 48 per cent gave an identical reading on each occasion. In another 48 per cent the differences were so small that the final assessment could be considered comparatively reliable. In 4 per cent the differences were so great that a new test was considered necessary.

144 sera of healthy subjects gave a negative reading at each of four tests made on the sera.

When the sera of 1307 hospital patients were tested, each 1—3 times, 90 gave positive readings. These represented diseases and conditions in which serum protein changes and expressly a relative increase in globulins are known to occur.

When the sera of 22 patients with rheumatic fever and 55 patients with rheumatoid arthritis were tested, four times each, a positive result was obtained with 73 per cent of the former and

71 per cent of the latter. The result was found to be correlated in each case with the activity of the disease.

The results obtained from the sera of the patients and their comparison with the results obtained by means of electrophoretic protein analysis show that a serum lability phenomenon evidently is involved.

Many indicators suggest that the behaviour of the precipitate in sulphuric acid is governed by the water and alcohol bound to the precipitate.

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